

INTESTINAL ACTIVITIES OF TRYPSIN, LIPASE AND PHOSPHOLIPASE AFTER
A TEST MEAL - AN EVALUATION OF 474 EXAMINATIONS.

by

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Abstract

After indirect stimulation of the pancreas by means of a test meal the intestinal activities of trypsin were determined in 474 subjects, lipase in 117 and phospholipase in 57. Trypsin levels were subnormal in 88%, lipase levels in 80% and phospholipase levels in 81% of patients with chronic pancreatic disease. The outcome of repeated tests (trypsin) was completely consistent in 20 out of 22 patients. Calculation of ratios between the enzymes studied suggested that lipase was the enzyme most susceptible to pancreatic damage. Also in cases with celiac disease and after a Polya gastric resection the decrease of the intestinal lipase concentration were more marked than that of the other enzymes. In 9% of the cases with chronic pancreatic disease the diagnosis would have been overlooked if either lipase or trypsin had been determined as the sole enzyme. In clinical practice it is recommended to estimate at least two enzymes both because abnormal ratios may be of diagnostic value and because the two different groups of enzymes provide a mutual check of the secretory capacity of pancreatic enzymes. On the whole the test was found to be reliable, simple, physiological and inexpensive in terms of resources and is highly recommended as a routine test of pancreatic function.

Indirect stimulation of the pancreas by administration of a liquid test meal as described by Borgström et al. in 1957 (1) mimics the physiological intestinal phase of digestion and has been successfully used to assess pancreatic function (11). Among the enzymes determined in the Lundh test, trypsin has been widely used (11, 14) and is often the sole test. Other enzymes used are amylase (21), chymotrypsin (13), lipase (1, 2, 23, 24), phospholipase (5). There is, however, no unanimity about the type and number of pancreatic enzymes which should be estimated in order to provide the most reliable results.

The present paper reviews and compares the concentrations of trypsin, lipase and phospholipase in 474 unselected patients. The object of the study was to investigate the interrelationships among some of the pancreatic enzymes in duodenal aspirates obtained after a test meal in order to determine the most satisfactory measure for routine investigal use.

Material.

474 subjects were examined between 1972 - 1975 at the Laboratory for Pancreatic diseases, Department of Surgery, Lund. As shown in table I the patients retrospectively could be placed in different diagnostic groups in 341 cases. In 126 patients proven pancreatic disease was found, in 32 gastric surgery had prior been performed and in these latter cases there were no signs of diseases in other organs, in 44 cases intestinal disorders were diagnosed and in 10 diabetes mellitus was the sole diagnosis. In 81 cases no definite diagnosis was able to make or the notes not traced. Twentytwo patients were examined twice and in 26 cases the test was unsatisfactory due to technical failures.

Table I. Patients grouped according to clinical diagnosis.

CONTROLS	133
PANCREATIC CANCER	25
CHRONIC PANCREATITIS	42
PANCREATIC CYSTS	4
CYSTIC FIBROSIS	3
ST. P. AC. PANCREATITIS	52
GASTRIC SURGERY	32
CELIAC DISEASE	29
CROHN'S DISEASE	10
ULCERATIVE COLITIS	5
DIABETES MELLITUS	10
UNDEFINED DIAGNOSIS	81
NOTES NOT TRACED	
NO. OF PATIENTS WITH REPEATED TESTS	22
UNSATISFACTORY TESTS	26

Among the remaining 133 20 were healthy students and 113 were patients without signs of pancreatic disease, intestinal inflammatory disease or diabetes, and who had not undergone gastric surgery.

Diagnosis

In all cases of pancreatic cancer the diagnosis was confirmed at laparotomy or autopsy and verified histologically. Chronic pancreatitis was diagnosed at laparotomy (histology) in 25 cases. In all 42 cases angiography and pancreatic scanning was performed and in 16 cases ERCP (endoscopic retrograde cholangio-pancreaticography) or peroperative Wirsungiography. Determination of iso-amylase in serum was done in 11 cases (20). All patients had a typical history of chronic pancreatitis and 33 patients were addicted to alcohol overconsumption. In the 3 cases of cystic fibrosis the "sweat test" was positive. In all cases of celiac disease small intestinal biopsy specimens showed a typical picture and were deficient of intracellular enzymes.

Methods

The test was performed in fasting patients without pre-medication. A duodenal tube (Levine 12) was placed with the tip in the horizontal part of the duodenum under fluoroscopic control. A testmeal according to Lundh (11) was thereafter given and the sampling started. During the first hour the duodenal aspirates were sampled in 10 minutes fractions and during the second hour in 30 minutes fractions. From each of the 8 fractions 1 ml was withdrawn after shaking for 1-2 minutes and pooled. All collections were performed on ice and stored at -20°C until analyzed.

In 117 of the patients lipase was determined and in 57 phospholipase while trypsin was estimated in all 474 patients. The enzyme determinations which were done in duplicate were performed only in the pooled aspirates. Trypsin was assayed by automatic titration using TAME (β -toluene-sulphonyl-L-arginine methyl ester) as the substrate (8). Lipase was determined by a scaled down version of the method described by Erlanson and Borgström (4) and phospholipase according to Ihse and Arnesjö (9).

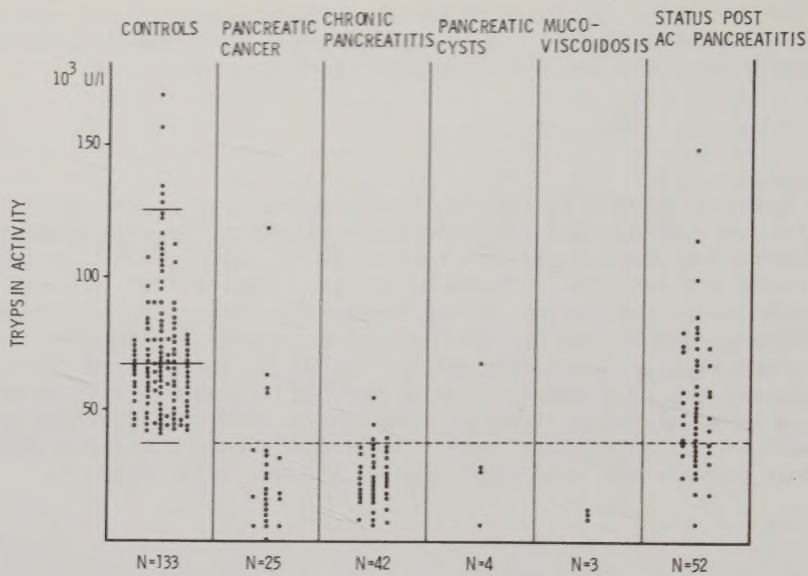
Statistical significances were calculated by means of the Student's t-test.

Results

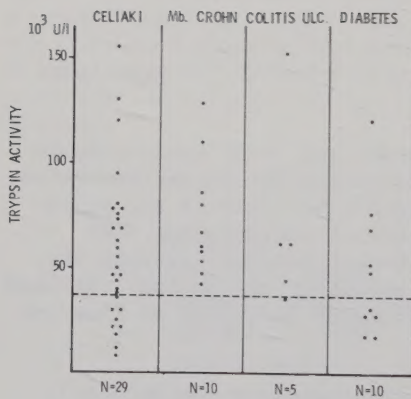
Trypsin

In Fig. 1 A-C the trypsin concentrations of all patients can be seen.

1 A



1 B



1 C

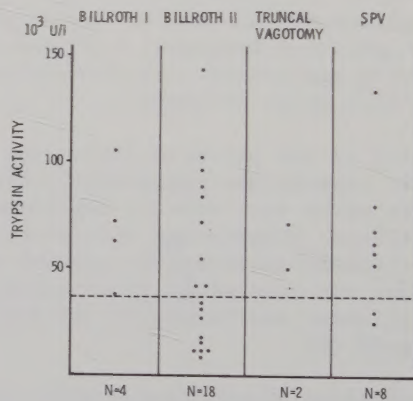


Fig. 1 A, 1 B and 1 C. Trypsin activity in pooled aspirates of controls and of patients with different gastrointestinal disorders. The lower level of normals (broken line) is mean - 2 S.D. Activity expressed as μ mole TAME released per ml per min.

2 A

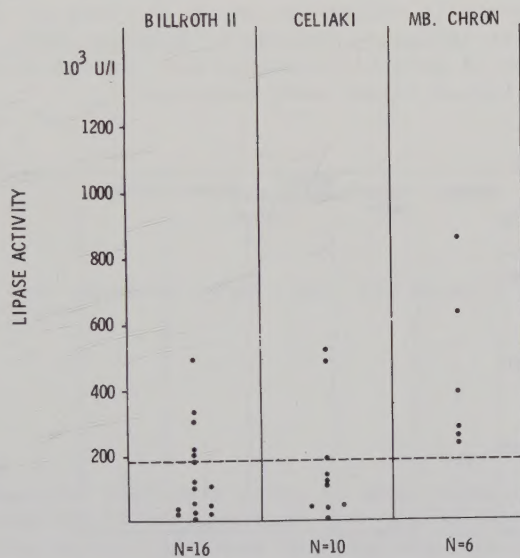
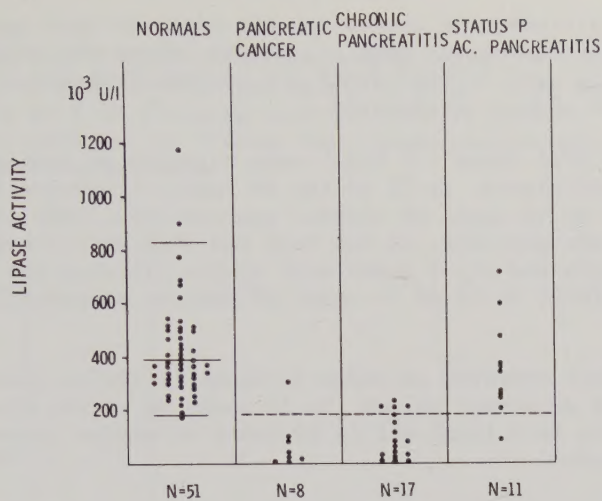


Fig. 2 A and 2 B. Lipase activity in pooled aspirates of controls and of patients with different gastrointestinal disorders. The lower level of normals (broken line) is mean - 2 S.D. Activity expressed as μ mole fatty acids released per ml per min.

When frequency distribution curves for the controls were constructed it was noted that the curves were log normal. Hence the ranges (mean $\pm$ 2 S.D.) finally used in this study are derived from the log normal data of the mean enzymic activities.

Values below 37 U/ml (mean - 2 S.D.) were regarded as indicating pancreatic insufficiency. In 21 of the 25 cases of pancreatic cancer (84%) and in 38 or 42 cases of chronic pancreatitis (90%) the Lundh test (trypsin) was positive. As the test was also positive in 3 of 4 pancreatic cysts and all 3 cases with cystic fibrosis the test all over was helpful in 65 of 74 cases of chronic pancreatic disease (88%).

Of the 18 patients operated ad modum Billroth II (Polya gastric resection) 9 had subnormal values. In 29 cases of celiac disease the test was positive in 9 (31%) and in 10 cases of severe juvenile diabetes in 5 cases.

Lipase

Lipase concentration from 117 patients are demonstrated in Fig. 2 A-B. Values below 185 U/ml (mean - 2 S.D.) were regarded as subnormal. In 7 of 8 cases of pancreatic cancer and 14 of 17 cases of chronic pancreatitis the test indicated pancreatic disease (80%). In 11 of 16 cases of Billroth II gastric resection and in 7 of 10 cases of celiac disease the lipase values were subnormal.

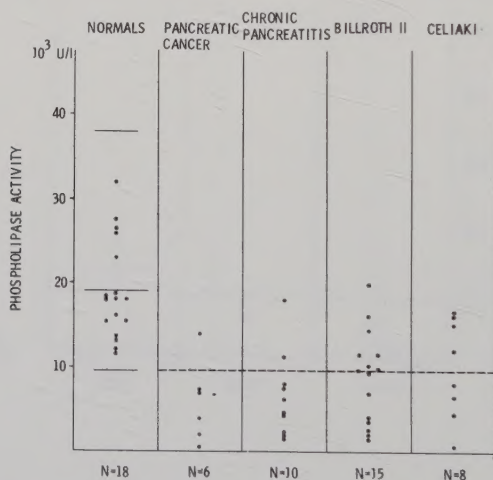


Fig. 3. Phospholipase activity in pooled aspirates of controls and of patients with different gastrointestinal disorders. The lower level of normals (broken line) is mean - 2 S.D. Activity expressed as μ mole fatty acids released per ml per min.

Phospholipase

Fig. 3 shows the 57 cases in whom phospholipase was determined. In 13 of 16 cases with chronic pancreatic disease (81%) the test was positive and in 8 of 15 cases with Billroth II gastric resection and 4 of 8 cases with celiac disease the lipase concentrations were below the lower level of normals.

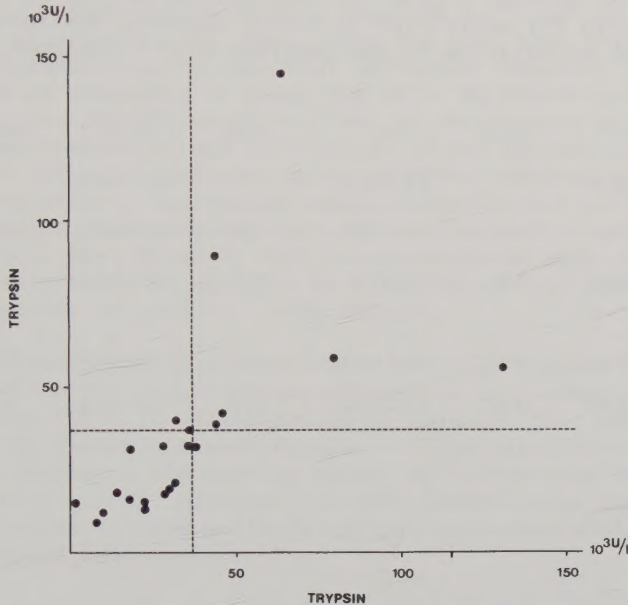


Fig. 4. Results of repeated Lundh test. For details see fig. 1.

Repeated tests

In 22 cases repeated test were done, in most cases because there was a suspicion from the clinicians that the results were inadequate. Fig. 4 shows the results of these examinations. As can be seen the test was positive at both occasions in 14 cases and normal at both occasions in 6 cases. Only 2 patients with border line values were the results of the test different at the two examinations.

Ratios between the enzymes

Table II shows the ratios between the different enzyme concentrations in normals and in some relevant diagnoses. The L/T and L/Ph ratios

were different in normals at the one hand and in patients with pancreatic cancer, chronic pancreatitis, celiac disease and those operated with Billroth II gastric resections at the other hand. The Ph/T ratio, however, was the same in normals as in the different diagnostic groups.

Table II. Ratios between lipase (L), phospholipase (Ph) and trypsin (T) activities in pooled duodenal aspirates after stimulation of the pancreas with a testmeal.

	L/T-ratio	P-value	L/Ph	P-value	Ph/T-ratio	P-value
Normals	5.84 ± 0.34 (50)		25.76 ± 2.38 (17)		0.28 ± 0.04 (18)	
B II-resection	2.48 ± 0.38 (16)	< 0.001	15.96 ± 2.81 (15)	< 0.02	0.18 ± 0.03 (15)	NS
Pancreatic cancer	2.89 ± 0.68 (8)	< 0.001	9.32 ± 2.80 (6)	< 0.001	0.25 ± 0.06 (6)	NS
Chronic pancreatitis	3.49 ± 0.62 (17)	< 0.005	11.96 ± 4.28 (9)	< 0.01	0.30 ± 0.06 (9)	NS
Celiaki	3.29 ± 0.61 (11)	< 0.001	11.54 ± 2.80 (6)	< 0.001	0.28 ± 0.09 (6)	NS

Pancreatic exocrine insufficiency was found in 98 patients. Among these patients the lipase activity but not the trypsin activity was decreased in 7 cases (7%) and the lipase within normal ranges but the trypsin decreased in 2 cases (2%). Hence in 9% of the cases with chronic pancreatic insufficiency the diagnosis would have been overlooked if only one of the two enzymes had been estimated.

Discussion

The present paper summarizes the experiences of almost 500 pancreatic function tests according to Lundh. Beside trypsin also lipase and phospholipase were determined.

The test was of practical values in the management of patients with chronic pancreatic disease in 88% when trypsin was estimated, in 80% when lipase was estimated and in 81% when phospholipase was estimated. During the last years several papers have been written dealing with the value of the Lundh test (for references see 10). Grouped together the test (trypsin) was of value in 86% of the cases of these patients.

Following gastric resection (Polya resection) the trypsin concentrations after test meal stimulation was subnormal in 50% (9/18), the lipase concentrations in 69% (11/16) and the phospholipase concentration in 53% (8/15) of the cases. Our results like those of Worning et al. (23) indicate that following a test meal the decrease of lipase seems to be more marked than that of other enzymes in patients operated on with gastric resection.

In celiac disease the pancreatic function is normal as measured by hormonal stimulation (3). When using the test meal stimulation the enzyme concentrations in duodenal aspirates, however, have been reported to be subnormal in about 30% (24). We found that the trypsin concentration was decreased in 9 out of 29 patients with celiac disease, the lipase concentrations in 7 out of 10 and phospholipase concentration in 4 out of 8 patients. As in patients operated upon with a Polya gastric resection, the lipase concentration in celiac disease, seems to be reduced more markedly than the concentrations of other enzymes studied (23, 24). Possibly this non-parallelism might reflect an altered pancreatic stimulation by gastrointestinal hormones in these two categories of patients (vide infra).

In a previous paper we reported that salivary amylase is often a major part of the total amylase determined in duodenal aspirates after a test meal (19). It was therefore recommended that pancreatic iso-amylases be determined specifically - if amylase assays is used - in order to eliminate the masking effect of the varying contribution from the salivary glands. Hospitals without laboratories capable to discriminate pancreatic from salivary amylase were thus advised to measure other enzymes than amylase.

In the present paper the concentration of lipase, phospholipase and trypsin are compared (Table II). It is obvious that the L/T as well as the L/Ph ratios are different in health and disease. The Ph/T ratio, however, is the same in normals as in patients. This might theoretically be related to the fact that trypsin is necessary for the activation of phospholipase but not of lipase but might also reflect a non parallel secretion of lipase as compared with the other two enzymes studied in pancreatic insufficiency. Worning and Müllertz (22) found considerable variation between the concentrations of different enzymes in response to a test meal in man. Sarles et al. (17) noted that lipase disappeared from human duodenal contents during prolonged infusion of secretin without change of the activities of the other enzymes and Ribet (15) noted an increase in the ratio of trypsin to lipase when pancreozymin was added to an infusion of secretin. Similar findings were made by Goldberg and Wormsley (6). According to Schön (18) and Rich (16) lipase seems to be the enzyme most susceptible to pancreatic damage. These reports, as well as the results presented in this paper question Babkin's hypothesis that pancreatic enzymes under all conditions are secreted in parallel.

In the present study 9% of the cases with chronic pancreatic disease would have been overlooked if either trypsin or lipase had been determined as the sole enzyme. We further found that the relationship between lipase at the one hand and trypsin and phospholipase at the other hand is different in cases with pancreatic insufficiency as compared to normals. In a previous study we found that pancreatic iso-amylase had a slightly better discriminatory ability discerning chronic pancreatic disease from normals than had trypsin after testmeal stimulation (20).

We believe, that in routine tests of pancreatic function at least two enzymes should be measured both because an abnormal ratio may be of diagnostic significance and because the two different groups of enzymes provide a mutual check of the secretory capacity of pancreatic enzymes. According to our opinion either pancreatic amylase-lipase or trypsin-lipase are suitable enzyme constellations in routine test meal tests.

The Lundh test is frequently criticized on the grounds that it is not a function test in physiological terms because the pancreas is stimulated indirectly. It is therefore important to determine whether under clinical conditions the tests is as useful as the secretin test in the diagnosis of pancreatic diseases. Several authors have found that it really is (7, 12, 25). Therefore, as the Lundh test is simple, physiological and inexpensive in terms of resources it is likely to be more useful than the secretin test in clinical practice because of its practical advantages.

Acknowledgement

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